

OPIC
OFFICE DE PROPRIÉTÉ
INTELLECTUELLE DU CANADA



CIPO
CANADIAN INTELLECTUAL
PROPERTY OFFICE

(12)(19)(CA) **Brevet-Patent**

(11)(21)(C) **2,079,205**

(22) 1992/09/25

(43) 1994/03/26

(45) 1998/02/10

(72) Falk, Rudolf Edgar, CA
(72) Asculai, Samuel Simon, CA
(72) Turley, Eva Anne, CA
(73) Hyal Pharmaceutical Corporation, CA
(51) Int Cl.⁶ A61K 31/73

(54) **UTILISATION DE L'ACIDE HYALURONIQUE ET DE
FORMULES POUR EMPECHER LA RESTENOSE
ARTERIELLE**

(55) **USE OF HYALURONIC ACID AND FORMS TO PREVENT
ARTERIAL RESTENOSIS**

COPY

(57) L'administration d'une quantité thérapeutiquement efficace et non toxique d'acide hyaluronique et/ou de sels de ce dernier et/ou d'homologues, analogues, dérivés complexes, esters, fragments et sous-unités d'acide hyaluronique permet de prévenir le rétrécissement des parois tubulaires chez un animal dont les parois tubulaires ont subi un traumatisme.

(57) For the prevention of the narrowing of the tubular walls of an animal after the tubular walls have been traumatized, the administration of a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid to the animal to prevent narrowing of the tubular walls.

BEST AVAILABLE COPY



Industrie Canada Industry Canada

ATTORNEY DOCKET NUMBER: 10177-191-999

SERIAL NUMBER: 10/603,115

REFERENCE: B10

3141

OPIC
OFFICE DE PROPRIÉTÉ
INTELLECTUELLE DU CANADA



CIPO
CANADIAN INTELLECTUAL
PROPERTY OFFICE

(12)(19)(CA) **Brevet-Patent**

(11)(21)(C) **2,079,205**

(22) 1992/09/25

(43) 1994/03/26

(45) 1998/02/10

(72) Falk, Rudolf Edgar, CA
(72) Asculai, Samuel Simon, CA
(72) Turley, Eva Anne, CA
(73) Hyal Pharmaceutical Corporation, CA

(51) Int Cl. A61K 31/73

(54) **UTILISATION DE L'ACIDE HYALURONIQUE ET DE
FORMULES POUR EMPECHER LA RESTENOSE
ARTERIELLE**

(54) **USE OF HYALURONIC ACID AND FORMS TO PREVENT
ARTERIAL RESTENOSIS**

COPY

(57) L'administration d'une quantité thérapeutiquement efficace et non toxique d'acide hyaluronique et/ou de sels de ce dernier et/ou d'homologues, analogues, dérivés complexes, esters, fragments et sous-unités d'acide hyaluronique permet de prévenir le rétrécissement des parois tubulaires chez un animal dont les parois tubulaires ont subi un traumatisme.

(57) For the prevention of the narrowing of the tubular walls of an animal after the tubular walls have been traumatized, the administration of a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid to the animal to prevent narrowing of the tubular walls.



Industrie Canada Industry Canada

2079205

TITLE OF INVENTION

USE OF HYALURONIC ACID AND FORMS TO PREVENT ARTERIAL
RESTENOSIS

FIELD OF INVENTION

5 This invention relates to the prevention of the narrowing (stenosis) of tubular walls of an animal after the tubular walls have been traumatized. In one embodiment, this invention relates to the prevention of arterial restenosis after balloon angioplasty.

BACKGROUND OF THE INVENTION

10 Balloon angioplasty is a widely accepted method of opening blockages in the coronary arteries. However in some patients after successful treatment by balloon angioplasty, arterial restenosis occurs. This time however the narrowing of the inner diameter (ID) of the artery is caused by growth (proliferation) of endothelial cells in the areas of irritation caused by the balloon
15 angioplasty. Thus reblockage occurs not by cholesterol build-up but by build up of endothelial cells on the inner wall of the artery reducing the inner diameter (ID) of the artery leading to an infarct. This narrowing of the inner diameter (ID) of tubular walls or proliferation of cells is not however restricted or limited to the coronary arteries. It can also occur post operatively causing restenosis in for
20 example peripheral vascular systems.

A number of proposals have been made in the prior art to prevent restenosis.

25 U.S. Patent 5,067,244 (Wolinsky et al.) purports to teach the use of a catheter having an inelastic balloon at one end thereof, where the balloon has minute perforations and contains a concentrated heparin solution which will be released through the perforations contacting an area of the artery after angioplasty to prevent restenosis.

U.S. Patent 5,116,864 (Hathaway et al.) purports to teach the prevention of restenosis in peripheral or cardiac vascular systems after vascular

recanalisation by systemic administration of photo activatable psoralen to give serum psoralen levels which inhibit smooth muscle cell growth.

U.S. Patent 5,092,841 (Spears, J.R.) purports to teach the treatment of an arterial wall injured during angioplasty by delivering bio-protective material between the wall and the angioplasty catheter so that the bio-protective material is entrapped and permeates into the tissues and vessels of the arterial wall during opposition of the angioplasty catheter.

EP 356275-A (Petitou et al.) purports to teach the use of new o-acylated glycosamino-glycan derivatives in the inhibition of post-operative restenosis.

Berk, B.C. et al in the J. Am. Coll. Cardiol. dated 1991 Vol. 17 #6 Supplement B, pp 111B-117B purports to discuss the pharmacologic roles of heparin and glucocorticoids to prevent restenosis after coronary angioplasty.

WO 9209561 (Utoh et al.) purports to teach the use of new ACAT inhibiting amide derivatives in treatment of restenosis after percutaneous transluminal coronary angioplasty.

WO 9208472 (Scarborough et al.) purports to teach the use of platelet antiadhesive peptide(s) obtained from snake venom for the prevention of restenosis following angioplasty.

WO 9207852 (Bovy et al.) purports to teach the use of certain biphenylalkyl xanthine derivatives to prevent post-angioplasty restenosis.

WO 9205782 (Pill, J.) purports to teach the use of thromboxane-A2-receptor antagonists (I) in the preparation of medicaments for inhibition of proliferative developments in obstructive vascular disorders ie. arterial restenosis.

WO 9118639 (GAJ et al.) purports to teach the inhibition of stenosis after balloon angioplasty, by the administration of fibronectin by continuous or bolus infusion, or by direct infusion into the stenotic region via the angioplasty catheter.

CA 2,042,159 laid open application (Ondetti, et al.) purports to teach the use of ACE inhibitor (via the oral or parenteral route) for preventing or reducing the risk of restenosis following angioplasty.

U.S. 4,929,602 (Harker, et al.) purports to teach a method of
5 inhibiting arterial restenosis by administration of D-phenyl alanyl-prolyl-arginyl-balomethyl ketone peptide derivative or a hydrolaltn acid addition thereof.

U.S. 4,820,732 (Shell, et al.) purports to teach a composition containing a prostaglandin compound for the reduction of restenosis and abrupt stenosis.

10 Applicant is also aware of a company Glycomed developing a fragment of Heparin that prevents arterial restenosis after balloon angioplasty.

In the basic research efforts in the latter 70s and the early 80's, there existed considerable confusion as to what role immunotherapy should take in cancer. Activation or "hyping" of macrophages was thought to be important.
15 However, in an examination by Romanis and Falk of peritoneal macrophages obtained from patients with neoplastic disease, there was definite evidence that these macrophages were already activated yet were co-existing with cancer cells and not causing their destruction.

It has been shown by several independent investigators that the
20 malfunction of macrophages or the putitive block is due to excessive prostaglandin and that this can be altered in tissue culture by corticosteroids, ASA, and the non-steroidal anti-inflammatory drugs, i.e. indomethacin, and naproxen (NaprosynTM). Again, in animal tumors it was repeatedly demonstrated that these substances could alter the response to neoplastic cells
25 and that various combinations of these substances employed with immune enhancing agents could produce very credible success in eliminating experimental tumors. Lala and co-workers combined Indomethacin therapy with Interleukin 2 and showed that this could effect a cure with experiment neoplasm.

There were continued problems with the use of any of these agents in the actual human in vivo experience. All of the non-steroidal anti-inflammatory agents (NSAID) produced major toxicity in terms of gastrointestinal, neurological, and other areas. Thus, the basis of the present approach is that under general circumstances the use of these agents in human disease, in sufficient amounts, the drug will penetrate to any pathological tissue to alter therapeutically local prostaglandin production. While intravenous preparations exist of indomethacin and now of other agents, the data is overwhelming, as is our own experience, that using these drugs alone produces prohibitive side effects in human subjects. Therefore only insufficient amounts can be brought into the body to effect more than occasional responses in neoplasm.

However the majority of the evidence is present to indicate and therefore it can be postulated that the basis for neoplastic development and how the initial cell "sneaks by" the immune surveillance mechanism relates to its production of prostaglandin. One need postulate only one mutation to alter the amount of prostaglandin synthesis produced by cells when they become "malignant" to establish a mechanism of blocking out the initial cell in any immune reaction, i.e. the macrophage. It therefore became essential to develop a combination of NSAIDS for clinical use to produce a major improvement in response in neoplastic disease and other conditions where excessive prostaglandin synthesis represents the basis of the pathogenesis of this disease state, i.e. arthritis, and various others of the so-called connective tissue inflammatory disorders and/or auto-aggressive diseases.

See also:

1. Modulation of Immunity in Cancer Patients by Prostaglandin Antagonists, Immunity to Cancer II, Alan R. Liss, Inc; and
2. Goodwin, J.S. (1981) Prostaglandin E and Cancer Growth Potential for Immunotherapy with Prostaglandin Synthesis Inhibitors, Augmentive Agents in Cancer Therapy, Raven Press, New York.

It is therefore an object of this invention to provide a method of treatment and formulations and pharmaceutical compositions for preventing arterial restenosis after balloon angioplasty when endothelial cell proliferation occurs on the inner arterial wall caused by irritation to the cells by balloon angioplasty.

It is a further object of the invention to provide such treatment using hyaluronic acid which is safe and essentially non-toxic.

Further and other objects of the invention will be realized by persons skilled in the art from the following summary of the invention and discussion with respect thereto.

SUMMARY OF THE INVENTION

Applicants believe that forms of hyaluronic acid (especially hyaluronic acid and salts thereof) will prevent stenosis of the inner diameter (ID) of irritated tubular walls and particularly prevent restenosis of the arterial walls by for example the proliferation of endothelial cells as a result of irritation arising from balloon angioplasty. The forms of hyaluronic acid (for example hyaluronic acid and salts of hyaluronic acid) can be administered intravenously or by injection (in the case of direct injection of small amounts) in effective amounts of about 10mg/70kg person to in excess of 3000mg/70kg person.

Therefore according to one aspect of the invention, there is provided a process for the prevention of the narrowing of the tubular walls of an animal after the tubular walls have been traumatized (for example wherein the tubular walls are arteries which have been subjected to balloon angioplasty) the process comprising the administration of a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments and subunits of hyaluronic acid to the animal to prevent narrowing of the tubular walls. Preferably the form of hyaluronic acid is hyaluronic acid and salts thereof. The amount of the form of hyaluronic acid administered is preferably between about 10mg/70kg person and

about 3000mg/70kg person.

Thus according to another aspect of the invention, a process is provided for the prevention of arterial restenosis after balloon angioplasty in a human, the process comprising the administration of a therapeutically effective
5 non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid to the human to prevent arterial restenosis. Once again preferably the form of hyaluronic acid is hyaluronic acid and salts thereof and preferably the amount of the form of hyaluronic acid administered is between about 10mg/70kg person
10 and about 3000mg/70kg person.

The compositions are preferably administered intravenously in a liquid form and include suitable diluents or other adjuvants as required for administration. With respect to small amounts to be administered, they may be administered by injection preferably at or proximate the site to be treated.

15 A therapeutically effective amount of a non-steroidal anti-inflammatory drug (NSAID) for enhancing the effect of the form of hyaluronic acid administered in the prevention of the narrowing of the tubular walls may be administered with the form of the hyaluronic acid. The addition of the non-steroidal anti-inflammatory agent will enhance the activity of the hyaluronic
20 acid in preventing the narrowing of the tubular walls for example enhancing the arterial restenosis prevention effect of the administered hyaluronic acid and/or salts thereof. The NSAID may be an NSAID suitable for the purposes and which may comprise Diclofenac, Indomethacin (solubilized in N-Methyl Glucamine), Piroxicam, the tromethamine salt of Ketorolac, acetylsalicylic acid, Naproxen and
25 the like. The amounts of NSAID may be appropriate accepted doses preferably administered to patients. In some cases dose amounts up to 10mg of the NSAID/kg of body weight (for example 1-2mg of NSAID/kg of body weight) are suitable. With Diclofenac much larger amounts are appropriate. Where greater than normal amounts of NSAIDS are used, in order to reduce side effects caused

by excess NSAID administration, greater than about 200mg of the form of Hyaluronic Acid (HA) per 70kg person may be administered to reduce and eliminate the side effects such as gastro-intestinal distress, neurological abnormalities, depression, etc.

- 5 A therapeutically effective amount of Vitamin C may also be added to the composition to enhance the effect of the Hyaluronic Acid administered. Such amount may be up to 50 grams - 100 grams in a dosage as Vitamin C is soluble and is excreted by the kidney although much lower amounts are normally used. Preferably the composition comprises a form of hyaluronic acid,
10 specifically preferred hyaluronic acid and/or salts thereof, an NSAID and Vitamin C for administration for the prevention of the narrowing of the tubular walls (for example the prevention of arterial restenosis after balloon angioplasty).

- Thus according to another aspect of the invention, the use of a pharmaceutical composition for the prevention of the narrowing of the tubular
15 walls of an animal after the tubular walls have been traumatized is provided, the use being of a pharmaceutical composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in association with a suitable diluent or pharmaceutically acceptable carrier
20 or other adjuvants to prevent narrowing of the tubular walls. Preferably the form of hyaluronic acid is hyaluronic acid and salts thereof.

- According to another aspect of the invention, the use of a pharmaceutical composition for the prevention of arterial restenosis after balloon angioplasty in a human is provided, the use being of a pharmaceutical
25 composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in association with a suitable diluent, pharmaceutically acceptable carrier or other adjuvants to prevent arterial restenosis preferably the form of hyaluronic acid is selected from

hyaluronic acid and salts thereof and the amount of the form of hyaluronic acid is between about 10mg/70kg person and about 3000mg/70kg person.

In one embodiment the form of the pharmaceutical composition is for intravenous administration.

- 5 According to still another aspect of the invention, the pharmaceutical composition comprises a therapeutically effective amount of non-steroidal anti-inflammatory drug (NSAID) for example Diclofenac, Indomethacin (solubilized in N-Methyl Glucamine), Piroxicam, the tromethamine salt of Ketorolac, acetylsalicylic acid and the like for enhancing the effect of the form of hyaluronic acid in the prevention of the narrowing of the tubular walls.

- Thus according to another aspect of the invention, the use of a pharmaceutical composition for the prevention of arterial restenosis after balloon angioplasty is provided, the use being of a pharmaceutical composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof in association with a suitable diluent or pharmaceutically acceptable carrier or other adjuvants to prevent arterial restenosis (by administration for example intravenously of the composition). In some embodiments the amount of the hyaluronic acid and/or salts thereof is between about 10mg/70kg person and about 3000mg /70kg person. The composition may further comprise a therapeutically effective amount of a non-steroidal anti-inflammatory drug (NSAID) for enhancing the arterial restenosis prevention effect of the administered hyaluronic acid and/or salts thereof administered. The NSAID may be at accepted appropriate doses depending on the NSAID for example up to about 10mg/70kg of body weight (for example 1-2mg of NSAID/kg of body weight). The appropriate dose for Diclofenac is much greater. Where it is desired to use a dose excess of NSAID, the amount of hyaluronic acid and salts thereof preferably exceeds about 200mg/70kg person.

The composition may further comprise a therapeutically effective

amount of Vitamin C for enhancing the effects of the form of hyaluronic acid to prevent narrowing of the tubular walls. The Vitamin C may be used in large amounts (for example even 50 - 100 grams) although much smaller amounts are suitable.

5 According to another aspect of the invention the use of:
 an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid,

10 in the manufacture of a pharmaceutical composition is provided for preventing the narrowing of the tubular walls of an animal after the tubular walls have been traumatized, the use being characterized by a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid being incorporated into the pharmaceutical composition and
15 being sufficient and effective to prevent the narrowing of the tubular walls which were traumatized as for example the arteries being damaged after balloon angioplasty. Preferably the form of hyaluronic acid is hyaluronic acid and/or salts thereof and the composition is in a liquid form. Preferably, the form of hyaluronic acid is utilized at a dose between about 10mg to about 3000mg/70kg
20 person and more preferably the form of hyaluronic acid is utilized at a dose greater than 200mg/70kg person.

 In one embodiment the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

 According to another aspect of the invention, the use of:
25 (1) hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, and

 (2) an agent selected from a non-steroidal anti-inflammatory drug (NSAID) and Vitamin C and combinations thereof is provided

in the manufacture of a pharmaceutical composition (including diluents, adjuvants and other carriers) for preventing the narrowing of the tubular walls of an animal after the tubular walls have been traumatized wherein a therapeutically effective amount of the hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid is administered to humans together with a therapeutically effective amount of the agent (2), the use being characterized in that the amount of component (1) is an effective amount to prevent the narrowing of the tubular walls of the animal and component (2) enhances the effect of component (1) in the prevention of the narrowing of the tubular walls.

Preferably component (2) is hyaluronic acid and/or salts thereof and the composition is in a liquid form (for example for intravenous use or injection). Preferably component (1) is utilized at a dose between about 10mg to about 3000mg/70kg person and more preferably component (1) is utilized at a dose greater than 200mg/70kg person.

Component 2 is utilized at amounts effective to enhance the effect of Component 1. Vitamin C may be utilized in amounts up to 50 - 100 grams per dose although much smaller amounts are more desirable. The NSAID can be administered in normally acceptable dose amounts depending on the NSAID. With some NSAIDS the amounts are 1-2mg of NSAID per Kg of body weight, in others up to about 10mg per kg bodyweight and in others such as Diclofenac, much larger amounts. Where the NSAID is used in dose excesses (greater amounts than the normally acceptable dose amounts, the amount of the form of hyaluronic acid exceeds about 200mg per 70kg person. Suitable NSAIDS are Diclofenac, Piroxicam, Indomethacin (solubilized in N-methyl glucamine), acetylsalicylic acid, \pm tromethamine salt of Ketorolac, naproxen and the like.

According to another aspect of the invention a pharmaceutical composition is provided comprising (together with diluents as required) an

effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, for preventing the narrowing of the tubular walls of an animal after the tubular walls have been traumatized, the composition being

5 characterized by an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid being incorporated into the pharmaceutical composition to prevent the narrowing of the tubular walls. Preferably the form of hyaluronic acid is hyaluronic acid and/or salts thereof and

10 preferably the composition is in a liquid form (for example an intravenous (I.V.) form in an I.V. bag with diluents and pharmaceutically acceptable carriers and adjuvants). The form of hyaluronic acid may be utilized at doses between about 10mg to about 3000mg/70kg person and preferably the form of hyaluronic acid is utilized at a dose greater than 200mg/70kg person (especially where dosage

15 excesses of NSAIDS are employed). In one embodiment the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

According to another aspect of the invention, a pharmaceutical composition is provided comprising (together with diluents, adjuvants and

20 other pharmaceutically acceptable carriers as and if desired);

(1) hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, and

(2) an agent selected from a non-steroidal anti-inflammatory

25 drug and Vitamin C and combinations thereof

for preventing the narrowing of the tubular walls of an animal after the tubular walls have been traumatized, the composition being characterized by an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits

of hyaluronic acid being incorporated into the composition together with a therapeutically effective amount of the agent (2), to prevent tubular wall narrowing, the composition being characterized that the amount of component (1) is an effective amount to prevent the narrowing of the tubular walls of the animal and the amount of component (2) enhances the effect of component (1) in the prevention of the narrowing of the tubular walls. Preferably component (1) is hyaluronic acid and/or salts thereof and preferably in a liquid dosage form, for example in an intravenous form (I.V. Bag). In some embodiments component (1) may be utilized at a dose between about 10mg to about 3000mg/70kg person. Preferably component (1) is utilized at a dose greater than 200mg/70kg person where dose excesses of the NSAID of component (2) are utilized. In one embodiment the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

Component 2 is utilized at amounts effective to enhance the effect of Component 1. Vitamin C may be utilized in amounts up to 50 - 100 grams per dose. The NSAID can be administered in appropriate dose amounts depending on the NSAID and if given in excess amounts the amount of the form of hyaluronic acid preferably exceeds about 200mg per 70kg person. Suitable NSAIDS are Diclofenac, Firoxicam, Indomethacin (solubilized in N-methyl glucamine), acetylsalicylic acid, \pm tromethamine salt of Ketorolac, naproxen and the like.

When the composition comprises an agent selected from NSAID and Vitamin C and combinations thereof, Applicants postulate that the hyaluronic acid and/or salts thereof and/or the homologues, analogues, derivatives, complexes, esters, fragments, and/or sub units of hyaluronic acid facilitate the transport of the agent to the site of irritation to enable the agent to penetrate the cells (in the artery, endothelial cells) which together will help prevent for example arterial restenosis.

By way of example and to illustrate the facilitation of the delivery or

transport of a chemical to a site in a mammal, when ethyl alcohol is injected directly into a cancer tumor, and sonographic (ultrasound) assessment is made, it is not dispersed throughout the tumor. When the ethyl alcohol to be administered into a tumor is carried by hyaluronic acid and/or salts thereof, 5 sonographic assessment of the tumor, demonstrates the dispersion of the ethyl alcohol throughout the tumor.

While Applicants postulate that the hyaluronic acid facilitates the transport and delivery, Applicants' invention may be used as described irrespective of the actual method of operation of the hyaluronic acid and/or salts thereof and/or the homologues, analogues, derivatives, complexes, esters, 10 fragments and sub units of hyaluronic acid with the NSAID and Vitamin C.

The combination of hyaluronic acid and salts thereof and other forms with different chemicals and drugs (Vitamin C, and NSAIDS) alters their distribution and performance in the human body and produces an unusual 15 targeting for underperfused tissue and/or pathological tissue. In this regard the use of ascorbic acid (Vitamin C) as a free radical scavenger (50 gm daily - 1000 times the daily dose in therapeutic purposes as a Vitamin) administered intravenously with 300 - 500mg of hyaluronic acid (sodium hyaluronate) immediately relieves bone pain and muscle pain and reduces inflammation in 20 cancer patients. The hyaluronic acid enhances the anti-neoplastic activity and effect of the ascorbic acid. It is thought that this enhanced activity eliminates the free radicals by acting as a free radical scavenger. In any event the patients feel better. This is also demonstrated with furosemide and hyaluronic acid where the activity of furosemide is enhanced only minimally when administered with 25 hyaluronic acid to a "normal" subject but the activity is enhanced significantly when administered to a patient whose kidney is underperfused or malfunctioning due to insufficient intra-vascular volume.

A similar situation occurs with the NSAIDS. As a major amount of soluble indomethacin is required, the chemical product was solubilized using

n-methyl glucamine at a dilution of 5mg/ml of n-methyl glucamine (NMG). This substance is then passed through a 22 micron Milipore™ filter to produce sterility. This material is non-toxic at 16 fold the therapeutic dose in animals and for this reason was considered appropriate to be used in human conditions.

5 Thus, Indocid™ solubilized in NMG is administered to human patients either into a tumor intraperitoneally, intrapleurally, or intravascularly at a varying dose up to 10 mg/kg where each dose of indomethacin is combined with 200 - 1000mg of hyaluronic acid (for example "LifeCore™" hyaluronic acid [sodium hyaluronate]) diluted in the original solution of indomethacin and NMG with
10 for example the "LifeCore™" hyaluronic acid. This produces an appropriate mixture and can be administered safely by any of the routes. [Similar clinical studies have been done with hyaluronic acid prepared by other methods, i.e. extraction. The extracted material is satisfactory to use for intratumor, intraperitoneal or intrapleural use with this substance.]

15 Thus when an NSAID for example indomethacin (dissolved in n-methyl glucamine) or other NSAID is administered with greater than 200mg hyaluronic acid for 1 - 2 mg/kg body weight of the NSAID (in one instance indomethacin and NMG), no major toxic side effects occur such as gastrointestinal distress, neurological abnormalities, depression, etc., even at elevated
20 amounts of indomethacin (if necessary). If the amount of hyaluronic acid is decreased below that amount, the usual side effects may begin to reoccur. In addition, the responses that have been observed are superior when the NSAID (for example Indocid™) is combined with hyaluronic acid demonstrating clearly that the combination is now "targeting" to the pathological tissue even when
25 administered by the systemic intravenous route. Thus, it has been observed that patients with neoplastic diseases when receiving in addition to other chemicals (for example ascorbic acid [Vitamin C], phloretin and anti-cancer drugs), 50 - 200 mg NSAID - hyaluronic acid (sodium hyaluronate) (for example indomethacin and hyaluronic acid) experience dramatic relief of pain immediately. This is

followed within a short period of time by a resolution and resorption of neoplastic lesions with an improvement of pulmonary, and liver function if there is tumor present in these organs. Thus the dead tumor material and the debris and tumor toxins appear to be better eliminated by the body through the action of the macrophages whose activity is enhanced by the addition of the NSAID (or a steroidal anti-inflammatory drug) administered with hyaluronic acid (or salt or other form thereof). Thus Applicants believe that the addition of the NSAID for example with hyaluronic acid (sodium hyaluronate) deblocks the macrophages by preventing enzymatic production of prostaglandin synthetase which blocks macrophage functioning. Thus the hyaluronic acid (and salt and other forms) not only enhance the activity of the NSAID but also reduce any side effects and toxicity that is associated with the use of the prostaglandin synthesis inhibitors.

The hyaluronic acid and salts thereof may be utilized at varying doses - 10 to 1000 mg/70 kg person. As there is no toxicity, the hyaluronic acid can obviously be administered in a dose excess (for example 3000 mg/70 kg individual) without any adverse effects.

One form of hyaluronic acid and/or salts thereof (for example sodium salt) and homologues, analogues, derivatives, complexes, esters, fragments, and sub units of hyaluronic acid, preferably hyaluronic acid and salts and thereof suitable for use with Applicant's invention is a fraction supplied by Serivet Laboratories Limited (now Hyal Pharmaceutical Corporation). One such fraction is a 15 ml vial of Sodium hyaluronate 20mg/ml (300mg/vial - Lot 2F3). The sodium hyaluronate fraction is a 2% solution with a mean average molecular weight of about 225,000. The fraction also contains water q.s. which is triple distilled and sterile in accordance with the U.S.P. for injection formulations. The vials of hyaluronic acid and/or salts thereof may be carried in a Type I borosilicate glass vial closed by a butyl stopper which does not react with the contents of the vial.

The fraction of hyaluronic acid and/or salts thereof (for example sodium salt) and homologues, analogues, derivatives, complexes, esters, fragments, and sub units of hyaluronic acid, preferably hyaluronic acid and salts thereof may comprise hyaluronic acid and/or salts thereof having the following

5 characteristics:

a purified, substantially pyrogen-free fraction of hyaluronic acid obtained from a natural source having at least one characteristic selected from the group consisting of the following:

- i) a molecular weight within the range of 150,000-225,000;
- 10 ii) less than about 1.25% sulphated mucopoly-saccharides on a total weight basis;
- iii) less than about 0.6% protein on a total weight basis;
- iv) less than about 150 ppm iron on a total weight basis;
- v) less than about 15 ppm lead on a total weight basis;
- 15 vi) less than 0.0025% glucosamine;
- vii) less than 0.025% glucuronic acid;
- viii) less than 0.025% N-acetylglucosamine;
- ix) less than 0.0025% amino acids;
- x) a UV extinction coefficient at 257 nm of less than about 0.275;
- 20 xi) a UV extinction coefficient at 280 nm of less than about 0.25;

and

- xii) a pH within the range of 7.3-7.9. Preferably the hyaluronic acid is mixed with water and the fraction of hyaluronic acid fraction has a mean average molecular weight within the range of 150,000-225,000. More preferably
- 25 the fraction of hyaluronic acid comprises at least one characteristic selected from the group consisting of the following characteristics:

- i) less than about 1% sulphated mucopolysaccharides on a total weight basis;
- ii) less than about 0.4% protein on a total weight basis;

- iii) less than about 100 ppm iron on a total weight basis;
- iv) less than about 10 ppm lead on a total weight basis;
- v) less than 0.00166% glucosamine;
- vi) less than 0.0166% glucuronic acid;
- 5 vii) less than 0.0166% N-acetylglucosamine;
- viii) less than 0.00166% amino acids;
- x) a UV extinction coefficient at 257 nm of less than about 0.23;
- xi) a UV extinction coefficient at 280 nm of less than 0.19; and
- xii) a pH within the range of 7.5-7.7

10 Other forms of hyaluronic acid and/or its salts, and homologues, derivatives, complexes, esters, fragments and sub units of hyaluronic acid may be chosen from other suppliers, for example those described in prior art documents. In addition Applicants propose the use of sodium hyaluronate produced and supplied by LifeCore™ Biomedical, Inc. having the following specifications

15	<u>Characteristic</u>	<u>Specification</u>
	Appearance	White to cream colored particles
20	Odor	No perceptible odor
	Viscosity Average Molecular Weight	< 750,000 Daltons
25	UV/Vis Scan, 190-820nm	Matches reference scan
	OD, 260nm	< 0.25 OD units
	Hyaluronidase Sensitivity	Positive response
30	IR Scan	Matches reference
	pH, 10mg/g solution	6.2-7.8

	Water	8% maximum							
	Protein	< 0.3 mcg/mg NaHy							
5	Acetate	< 10.0 mcg/mg NaHy							
	Heavy Metals, maximum ppm								
	As	Cd	Cr	Co	Cu	Fe	Pb	Hg	Ni
	2.0	5.0	5.0	10.0	10.0	25.0	10.0	10.0	5.0
10	Microbial Bioburden	None observed							
	Endotoxin	< 0.07 EU/mg NaHy							
15	Biological Safety Testing	Passes Rabbit Ocular Toxicity Test							

The following references teach hyaluronic acid, sources thereof and processes of the manufacture and recovery thereof.

20 United States Patent 4,141,973 teaches hyaluronic acid fractions (including sodium salts) having:

- 25 (a) an average molecular weight greater than about 750,000, preferably greater than about 1,200,000 - that is, a limiting viscosity number greater than about 1400 cm³/g., and preferably greater than about 2000 cm³/g.;
- (b) a protein content of less than 0.5% by weight;
- (c) ultraviolet light absorbance of a 1% solution of sodium hyaluronate of less than 3.0 at 257 nanometers wavelength and
- 30 less than 2.0 at 280 nanometers wavelength;
- (d) a kinematic viscosity of a 1% solution of sodium hyaluronate in physiological buffer greater than about 1000 centistokes, preferably greater than 10,000 centistokes;

(e) a molar optical rotation of a 0.1 - 0.2% sodium hyaluronate solution in physiological buffer of less than -11×10^3 degree $\cdot \text{cm}^2/\text{mole}$ (of disaccharide) measured at 220 nanometers;

(f) no significant cellular infiltration of the vitreous and anterior chamber, no flare in the aqueous humor, no haze or flare in the vitreous and no pathological changes to the cornea, lens, iris, retina, and choroid of the owl monkey eye when one milliliter of a 1% solution of sodium hyaluronate dissolved in physiological buffer is implanted in the vitreous replacing approximately one-half the existing liquid vitreous, said HUA being

- (g) sterile and pyrogen free and
- (h) non-antigenic."

Canadian Letters Patent 1,205,031 (which refers to United States Patent 4,141,973 as prior art) refers to hyaluronic acid fractions having average molecular weights of from 50,000 to 100,000; 250,000 to 350,000; and 500,000 to 730,000 and discusses processes of their manufacture.

Where high molecular weight hyaluronic acid (or salts or other forms thereof) is used, it must be diluted to permit administration and ensure no intramuscular coagulation.

One formulation of Ascorbic Acid (Vitamin C) injection USP is manufactured by Steris Laboratories, Inc., Phoenix, Arizona, 85043 U.S.A. and comprises 22 mg/ml (equivalent to sodium ascorbate 250 mg/ml) in 30ml, 50ml, or 100ml individual containers, 30ml size being preferred.

As many changes can be made to the invention without departing from the scope of the invention, it is intended that all material contained herein be interpreted as illustrative of the invention and not in a limiting sense.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE AS FOLLOWS:

1. A method of preparing a pharmaceutical composition for use in the prevention of the narrowing of the tubular walls of an animal by the proliferation of endothelial cell growth in the area of trauma after the tubular walls have been traumatized, which method comprises incorporating a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in the composition to the animal to prevent narrowing of the tubular walls.
2. The method of Claim 1, wherein the form of hyaluronic acid is selected from hyaluronic acid and salts thereof, having a molecular weight less than 750,000 daltons.
3. The method of Claim 1, wherein the tubular walls are arteries which have been subjected to balloon angioplasty.
4. The method of Claim 1, 2 or 3, wherein the amount of the form of hyaluronic acid is between about 10mg/70kg person and about 3000mg /70kg person.
5. A method of preparing a pharmaceutical composition for use in the prevention of arterial restenosis after balloon angioplasty in a human, which method comprises incorporating a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in the composition to the human to prevent arterial restenosis.

2079205

6. The method of Claim 5, wherein the form of hyaluronic acid is selected from hyaluronic acid and salts thereof, having a molecular weight less than 750,000 daltons.
7. The method of Claim 5 or 6, wherein the amount of the form of hyaluronic acid is between about 10mg/70kg person and about 3000mg/70kg person.
8. The method of Claim 1, 2 or 3 wherein the composition is in intravenous form.
9. The method of Claim 5 or 6 wherein the composition is in intravenous form.
10. The method of Claim 1, 2 or 3 wherein the composition further comprises a therapeutically effective amount of a non-steroidal anti-inflammatory drug (NSAID) for enhancing the effect of the form of hyaluronic acid in the composition for use in the prevention of the narrowing of the tubular walls.
11. The method of Claim 5 or 6 wherein the composition further comprises an effective amount of a non-steroidal anti-inflammatory drug (NSAID) for enhancing the effect of the form of hyaluronic acid in the composition for use in the prevention of the arterial restenosis.
12. A method of preparing a pharmaceutical composition for use in the prevention of arterial restenosis after balloon angioplasty, which method comprises incorporating a therapeutically effective non-toxic amount of

hyaluronic acid and/or salts thereof in the composition to prevent arterial restenosis.

13. The method of Claim 12, wherein the amount of the hyaluronic acid and/or salts thereof is between about 10mg/70kg person and about 3000mg/70kg person, and the hyaluronic acid and/or salts thereof have a molecular weight less than 750,000 daltons.

14. The method of Claim 13 wherein the composition further comprises a therapeutically effective amount of an agent selected from a non-steroidal anti-inflammatory drug (NSAID) and Vitamin C and combinations thereof for enhancing the arterial restenosis prevention effect of the administered hyaluronic acid and/or salts thereof in the composition.

15. The method of Claim 13 wherein the composition further comprises an agent selected from a non-steroidal anti-inflammatory drug (NSAID) and Vitamin C and combinations thereof for enhancing the arterial restenosis prevention effect of the hyaluronic acid and/or salts thereof in the composition, the non-steroidal anti-inflammatory drug being selected in such amount to be effective in enhancing the arterial restenosis prevention effect of the hyaluronic acid and/or salts thereof in the composition.

16. The method of Claim 14 or 15 wherein the amount of the NSAID is a dose excess of the NSAID and the amount of hyaluronic acid and salts thereof exceeds about 200mg/70kg person.

17. The method of Claim 1, 2, 5, 6, 10, 11, 12, 13 or 16 wherein the composition further comprises an effective amount of Vitamin C for enhancing the effect of the form of hyaluronic acid in the composition for the prevention of

2079205

the narrowing of the tubular walls.

18. The use of a pharmaceutical composition for the prevention of the narrowing of the tubular walls of an animal by the proliferation of endothelial cell growth in the area of trauma after the tubular walls have been traumatized, the use being of a pharmaceutical composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in association with a suitable diluent or pharmaceutically acceptable carrier to prevent narrowing of the tubular walls.

19. The use of Claim 18, wherein the form of hyaluronic acid is selected from hyaluronic acid and salts thereof, having a molecular weight less than 750,000 daltons.

20. The use of Claim 18, wherein the tubular walls are arteries which have been subjected to balloon angioplasty.

21. The use of Claim 18, 19 or 20, wherein the amount of the form of hyaluronic acid administered is between about 10mg/70kg person and about 3000mg /70kg person.

22. The use of a pharmaceutical composition for the prevention of arterial restenosis after balloon angioplasty in a human, the use being of a pharmaceutical composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid in association with a suitable diluent or pharmaceutically acceptable carrier to prevent arterial restenosis.

23. The use of Claim 22, wherein the form of hyaluronic acid is selected from hyaluronic acid and salts thereof, having a molecular weight less than 750,000 daltons.

24. The use of Claim 23, wherein the amount of the form of hyaluronic acid is between about 10mg/70kg person and about 3000mg/70kg person.

25. The use of Claim 18, 19, 20, or 22, wherein the form of the pharmaceutical composition is for intravenous administration.

26. The use of any of the Claims 18, 19, 22, 23 or 24 further comprising a therapeutically effective amount of non-steroidal anti-inflammatory drug (NSAID) for enhancing the effect of the form of hyaluronic acid in the prevention of the narrowing of the tubular walls.

27. The use of a pharmaceutical composition for the prevention of arterial restenosis after balloon angioplasty, the use being of a pharmaceutical composition comprising a therapeutically effective non-toxic amount of hyaluronic acid and/or salts thereof in association with a suitable diluent or pharmaceutically acceptable carrier to prevent arterial restenosis.

28. The use of Claim 27, wherein the amount of the hyaluronic acid and/or salts thereof is between about 10mg/70kg person and about 3000mg /70kg person, and the hyaluronic acid and/or salts thereof has a molecular weight less than 750,000 daltons.

29. The use of Claim 27 or 28 further comprising an agent selected from a therapeutically effective amount of a non-steroidal anti-inflammatory drug

(NSAID) and Vitamin C and combinations thereof for enhancing the arterial restenosis prevention effect of the administered hyaluronic acid and/or salts thereof administered.

30. The use of Claim 27 or 28 further comprising an agent selected from a therapeutically effective amount of a non-steroidal anti-inflammatory drug (NSAID) and Vitamin C and combinations thereof for enhancing the arterial restenosis prevention effect of the hyaluronic acid and/or salts thereof administered, the non-steroidal anti-inflammatory drug and Vitamin C being selected in such amounts to be effective in enhancing the arterial restenosis prevention effect of the administered hyaluronic acid and/or salts thereof.

31. The use of Claim 29 or 30 wherein the amount of the NSAID is a dose excess of the NSAID and the amount of hyaluronic acid and salts thereof exceeds about 200mg/70kg person.

32. The use of Claim 18, 19, 22, 23, 24, 27 or 28, further comprising a therapeutically effective amount of Vitamin C for enhancing the effects of the form of hyaluronic acid to prevent narrowing of the tubular walls.

33. The use of
an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid,

in the manufacture of a pharmaceutical composition for preventing the narrowing of the tubular walls of an animal by the proliferation of endothelial cell growth in the area of trauma after the tubular walls have been traumatized, the use being characterized by an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives,

complexes, esters, fragments, and subunits of hyaluronic acid being incorporated into the pharmaceutical composition.

34. The use of Claim 33 wherein the form of hyaluronic acid is hyaluronic acid and/or salts thereof, having a molecular weight less than 750,000 daltons.

35. The use according to Claim 33 or Claim 34 in an intravenous form.

36. The use of Claim 35 wherein the form of hyaluronic acid is utilized at a dose between about 10mg to about 3000mg/70kg person.

37. The use of Claim 35 wherein the form of hyaluronic acid is utilized at a dose greater than 200mg/70kg person.

38. The use of Claim 36 or 37 wherein the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

39. The use of;

(1) hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, and

(2) an agent selected from a non-steroidal anti-inflammatory drug and Vitamin C and combinations thereof

in the manufacture of a pharmaceutical composition for preventing the narrowing of the tubular walls by the proliferation of endothelial cell growth in the area of trauma of an animal after the tubular walls have been traumatized wherein a therapeutically effective amount of the hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters,

fragments, and subunits of hyaluronic acid is administered to humans together with a therapeutically effective amount of the agent (2), the use being characterized in that the amount of component (1) is an effective amount to prevent the narrowing of the tubular walls of the animal and component (2) enhances the effect of component (1) in the prevention of the narrowing of the tubular walls.

40. The use of Claim 39 wherein component (2) is hyaluronic acid and/or salts thereof, having a molecular weight less than 750,000 daltons.

41. The use according to Claim 39 or Claim 40 in a liquid form.

42. The use of Claim 41, wherein component (1) is utilized at a dose between about 10mg/70kg person to about 3000mg/70kg person.

43. The use of Claim 41, wherein component (1) is utilized at a dose greater than 200mg/70kg person.

44. The use of Claim 42 or 43, wherein the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

45. A pharmaceutical composition comprising, together with suitable diluents, as required an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, for preventing the narrowing of the tubular walls of an animal by the proliferation of endothelial cell growth in the area of trauma after the tubular walls have been traumatized, the composition being characterized by an effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters,

A

fragments, and subunits of hyaluronic acid being incorporated into the pharmaceutical composition to prevent the narrowing of the tubular walls.

46. The composition of Claim 45 wherein the form of hyaluronic acid is hyaluronic acid and/or salts thereof, having a molecular weight less than 750,000 daltons.

47. The composition according to Claim 45 or Claim 46 in a liquid form.

48. The composition of Claim 47 wherein the form of hyaluronic acid is utilized at a dose between about 10mg/70kg person to about 3000mg/70kg person.

49. The composition of Claim 47 wherein the form of hyaluronic acid is utilized at a dose greater than 200mg/70kg person.

50. The composition of Claim 48 or 49 wherein the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

51. A pharmaceutical composition comprising together with diluents, adjuvants and other pharmaceutical carriers as desired;

(1) hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid, and

(2) an agent selected from a non-steroidal anti-inflammatory drug and Vitamin C and combinations thereof

for preventing the narrowing of the tubular walls of an animal by the proliferation of endothelial cell growth in the area of trauma after the tubular walls have been traumatized, the composition being characterized by an

effective non-toxic amount of hyaluronic acid and/or salts thereof and/or homologues, analogues, derivatives, complexes, esters, fragments, and subunits of hyaluronic acid being incorporated into the composition together with a therapeutically effective amount of agent (2), to prevent tubular wall narrowing, the composition being characterized that the amount of component (1) is an effective amount to prevent the narrowing of the tubular walls of the animal and component (2) enhances the effect of component (1) in the prevention of the narrowing of the tubular walls.

52. The composition of Claim 51 wherein component (1) is hyaluronic acid and/or salts thereof, having a molecular weight less than 750,000 daltons.

53. The composition according to Claim 51 or Claim 52 in a liquid form.

54. The composition of Claim 53, wherein component (1) is utilized at a dose between about 10mg to about 3000mg/70kg person.

55. The composition of Claim 54, wherein component (1) is utilized at a dose greater than 200mg/70kg person.

56. The composition of Claim 54 or 55, wherein the pharmaceutical composition is for prevention of arterial restenosis after balloon angioplasty in humans.

57. The composition of Claim 55, wherein agent (2) comprises a dose excess of NSAID.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☒ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.